NOAA Technical Memorandum NMFS-SEFC-214

PROCEEDINGS OF THE EIGHTH ANNUAL WORKSHOP ON SEA TURTLE CONSERVATION AND BIOLOGY

24 - 26 February 1988

Fort Fisher, North Carolina

Barbara A. Schroeder, Compiler

December 1988

STYROFOAM BOX AND BEACH TEMPERATURES IN RELATION TO INCUBATION AND SEX RATIOS OF KEMP'S RIDLEY SEA TURTLES

Donna J. Shaver
National Park Service
Padre Island National Seashore
9405 S. Padre Island Drive
Corpus Christi, TX 78418

David Wm. Owens
Texas A&M University
College Station, TX 77843

Allan H. Chaney
Texas A&I University
Kingsville, TX 78363

Charles W. Caillouet, Jr.
National Marine Fisheries Service
Galveston, TX 77551

Patrick Burchfield
Gladys Porter Zoo
Brownsville, TX 78520

Rene Marquez M.
Instituto Nacional de la Pesca
Manzanillo, Col. 28200, Mexico

The international program to restore and enhance the Kemp's ridley sea turtle (*Lepidochelys kempi*) population and establish a secondary breeding population of this species at Padre Island National Seashore (PAIS), Texas began in 1978. Each summer approximately 2,000 eggs (20 clutches) were collected at Rancho Nuevo (RN), Mexico, packed in Padre Island sand in styrofoam boxes and shipped to PAIS. The boxes were placed on shelves in a concrete building at Rancho Nuevo prior to shipment and then in a screen-enclosed shed at PAIS. Beginning in 1982, ambient shed and incubation box temperatures were commonly measured twice daily (usually maximum and minimum) and bihourly for a 24-hour period once a week at Rancho Nuevo and hourly each day at PAIS.

Incubating eggs of other sea turtle species in styrofoam boxes has, in some instances, subjected the eggs to slightly different thermal regimes than those occurring under natural conditions and had a masculinizing effect on the embryos. Wibbels et al (in prep.) found that 1978-1984 year-class *L. kempi* eggs incubated in styrofoam boxes produced males and females annually, but that males predominated much of the time (Table 1). They concluded that the average incubation temperature available during the critical sex determining period must have been slightly below the natural pivotal temperature (that which produces a 50:50 male to female sex ratio) of the population.

Table 1. Results of incubation and analysis of sex ratios of 1978 - 1987 year class Kemp's ridley sea turtle eggs sent to Padre Island National Seashore.

Year	Number eggs received	Percent hatched	Mean incubation period (days)	Number identified to gender[a]	Percent female
1978	2,191	88.1	51.5	32	34.4
1979	2,053	85.7	52.0	22	40.9
1980	2,976	84.1	50.5	0	-
1981	2,279	83.3	48.3	4	100.0
1982	2,017	77.6	51.0	94	31.9
1983	2,006	12.1	52.0[b]	12	50.0
1984	1,976	90.7	51.1	159	28.3
1985	1,978	84.1	48.8	156	53.8
1986	2,011	88.3	46.7	53	83.0
1987	2,001	64.3	47.6	516	99.6

[a]Using gonadal histology, necropsy, laparoscopy, tail length evaluation or serum testosterone assays
[b]Calculated based upon the 9 clutches that hatched

Temperatures for incubating eggs of the 1985-1987 year-classes were intentionally raised in an attempt to increase the proportion of females produced. At both RN and PAIS, eggs were placed on upper level shelves (as space permitted) where ambient temperatures were warmer than those recorded at lower shelf heights. The Rancho Nuevo egg house door was left closed and the windows were often covered. The walls of the PAIS incubation shed were covered with plastic sheeting and bamboo shades to trap heat and a heater was run nightly to moderate excessive fluctuations in temperatures. Mean ambient temperature at top shelf height in the PAIS shed was significantly lower during the 1984 incubation season (mean = 28.0°C, SD = 1.8°C, n = 116) than during the 1985 (mean = 31.1°C, SD = 2.8°C, n = 569) ($t = -15.1965$, $df = 683$, $P < 0.01$), 1986 (mean = 30.0°C, SD = 2.5°C, n = 945) ($t = -10.5013$, $df = 1059$, $P < 0.01$) and 1987 (mean = 30.0°C, SD = 2.3°C, n = 504) ($t = -9.9543$, $df = 618$, $P < 0.01$) seasons. Because incubation temperatures cycle with ambient temperatures, the recorded incubation temperatures of 1985-1987 were higher than those of previous years. The mean incubation period for clutches of the 1984 year-class was significantly longer than that for clutches of the 1985 ($t = 4.8049$, $df = 37$, $P < 0.01$), 1986 ($t = 12.2299$, $df = 39$, $P < 0.01$) and 1987 ($t = 6.7541$, $df = 37$, $P < 0.01$) year-classes (Table 1). Apparently the warmer incubation temperatures of the 1985-1987 year-class clutches shortened incubation periods and increased percentages of females produced. A

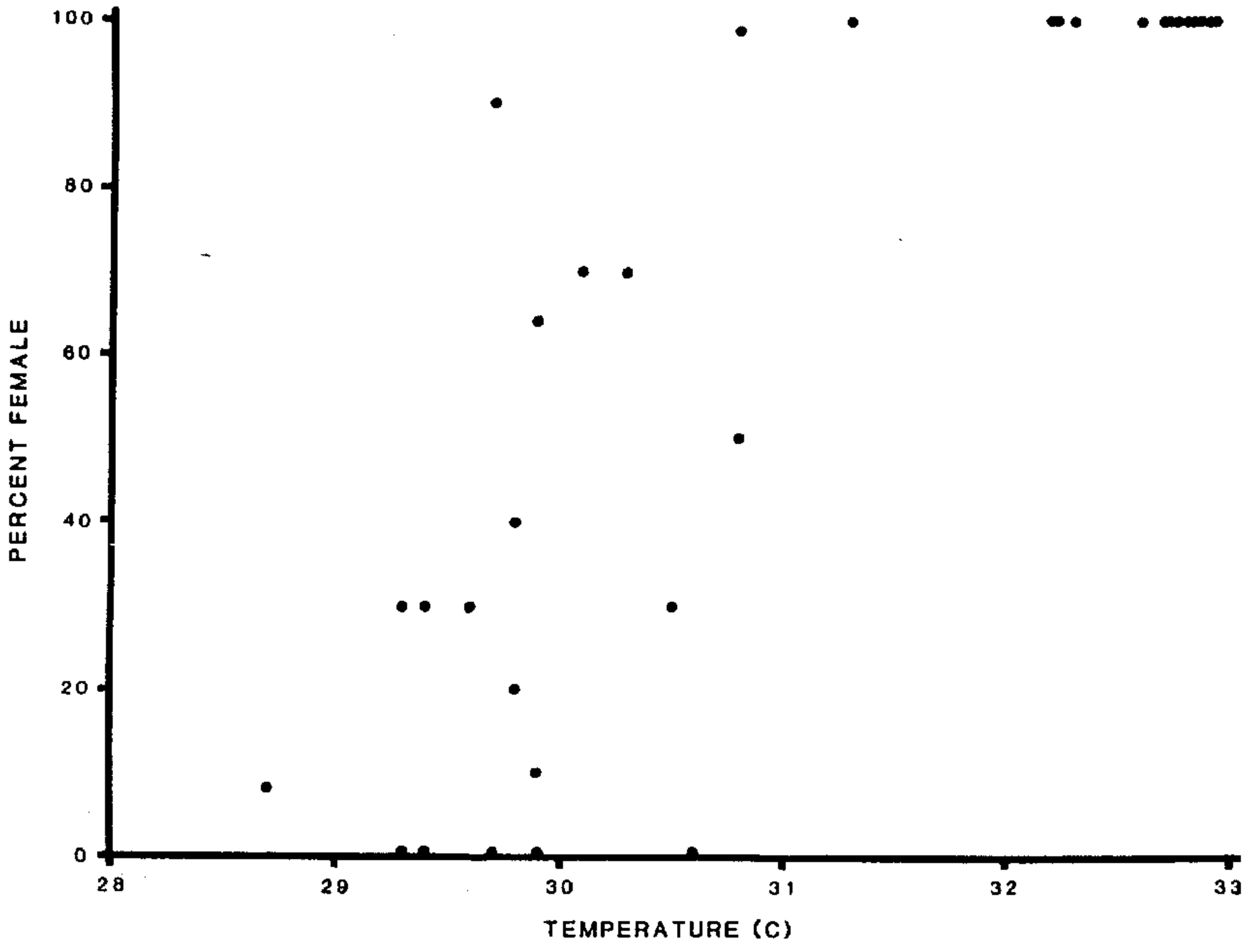

Figure 1. Kemp's ridley sea turtle mean middle third of incubation period temperatures in relation to percent of females from 1982 - 1987 clutches in which 10 or more individuals were positively identified to gender.

preponderance of the dead hatchlings, late-staged embryos and stranded yearlings examined from the 1985-1987 year-classes were identified as females. Of all specimens identified to gender, 53.8% of the 1985 year-class (n = 156), 83.0% of the 1986 year-class (n = 53), and 99.6% of the 1987 year-class (n = 516) were female.

Mean temperatures during the middle third of the incubation period and percent females for all 1982-1987 year-class clutches in which 10 or more individuals were positively identified to gender (n = 32) were correlated in an attempt to derive the first estimate of a pivotal temperature for Kemp's ridley (Figure 1). All clutches with mean temperatures exceeding 30.8°C produced100% females. However, a wide range of sex ratios were found in the clutches with lower mean temperatures. Some of the variation probably stems from lack of uniformity in the times that temperatures were recorded at Rancho Nuevo as well as differences in times that temperatures were taken at Rancho Nuevo and Padre Island. Bull (1985) found that the variance of incubation temperatures, as well as mean temperatures, influenced sex determination in *Graptemys* sp. Standora and Spotila (1985) stated that factors other than temperature, such as osmotic stress and O_2 and CO_2 levels, may influence sex within the critical range where a mixture of males and females are typically produced. Limpus et al. (1985) found that in *Caretta caretta* the pivotal temperature may vary from clutch to clutch. The scatter of our data points as well as general paucity of knowledge about the mechanism by which temperature influences sex in sea turtles affects the reliability of inferences drawn about pivotal temperatures for Kemp's ridley. However,

a preliminary analysis of the data on percent female vs. mean incubation temperature during the middle third of the incubation period was performed using only those data points associated with mean middle third temperatures below 31.5°C (n = 20). The best straight-line regression of percent female (Y) vs. mean temperature (X) was $Y = -1297.8747 + 44.7152X$, $r^2 = 0.68$, $P < 0.001$ for $H_o: B' = 0$. When variables were reversed, the equation was recalculated ($X = 29.4457 + 0.0151Y$, $r^2 = 0.68$, $P < 0.001$, $H_o: B' = 0$), from which the pivotal temperature (at which 50% females would be produced) was estimated to be 30.2°C with 95% confidence intervals from 29.9 to 30.5°C. Fitting a variety of sigmoid curves to the data did not improve the fit.

A study of beach temperature profiles was undertaken during the summer of 1986 to examine temperatures at which Kemp's ridley sea turtle eggs would incubate if laid at three locations along PAIS. These temperatures were to be compared with simultaneously measured temperatures at Rancho Nuevo. The three Padre Island sites were located approximately 30 km apart, numbered in ascending order from north to south. Thermocouple probes, placed in the same topographical areas at all study sites, were arranged in six rows ranging from the mid-beach to the middle upslope of the second foredune. Ambient, sand surface and 15, 30 and 45 cm substrate depth temperatures were recorded at PAIS but only ambient, sand surface and 30 cm depth (mid-nest depth) temperatures were monitored at Rancho Nuevo. Temperatures were monitored once a week for a 24-hour period, from noon to noon, with readings made once every 2 h. Simultaneous study dates were scheduled from mid-April to mid-August to collect data for the entire nesting and incubation season. Data were collected on all 18 study dates at PAIS and 11 of those dates at Rancho Nuevo.

During the period when most eggs would be undergoing their middle third of development, June and early July, in the preferred nesting areas (mid-beach to the top of the first foredune) at mid-nest depth, the temperatures at PAIS sites 2 and 3 and the Rancho Nuevo site were not appreciably different. Temperatures at site 1 on PAIS were only slightly cooler than temperatures at the other areas. The estimated pivotal temperature and 95% confidence intervals were plotted with the range of mean temperatures found along the entire beach width on each study date to predict seasonal trends in sex ratios (Figure 2). Clutches undergoing their middle third of incubation early in the nesting season at Rancho Nuevo should produce primarily males, later portions of the season primarily females and the middle of the season a mixture. This is similar to the pattern hypothesized by Standora and Spotila (1985) for this species. Based upon sand temperatures, a similar pattern is expected for the three sites on PAIS.

Evidently the degree of difference in temperatures between natural nests and styrofoam boxes varies with the species (natural nest depth) and individual recovery programs (hatchery conditions). Maximum 30 cm depth and styrofoam incubation box temperatures occurred between 2000-2400 h and minimum temperatures between 0800-1200 h at RN and PAIS. The duration of maximum and minimum temperatures (2-4 h) and daily range of temperatures (1-3°C) were very similar in styrofoam boxes and sand at mid-nest depth. At times, mean temperatures of eggs incubated in styrofoam boxes were higher or lower than those of sand at mid-nest depth. Direct correlations of styrofoam box and beach temperatures cannot be made with accuracy because of the wide range of temperatures available along the entire beach width, the effect of vegetative cover and local weather conditions and the production of metabolic heat by the embryos during the middle and last thirds of development.

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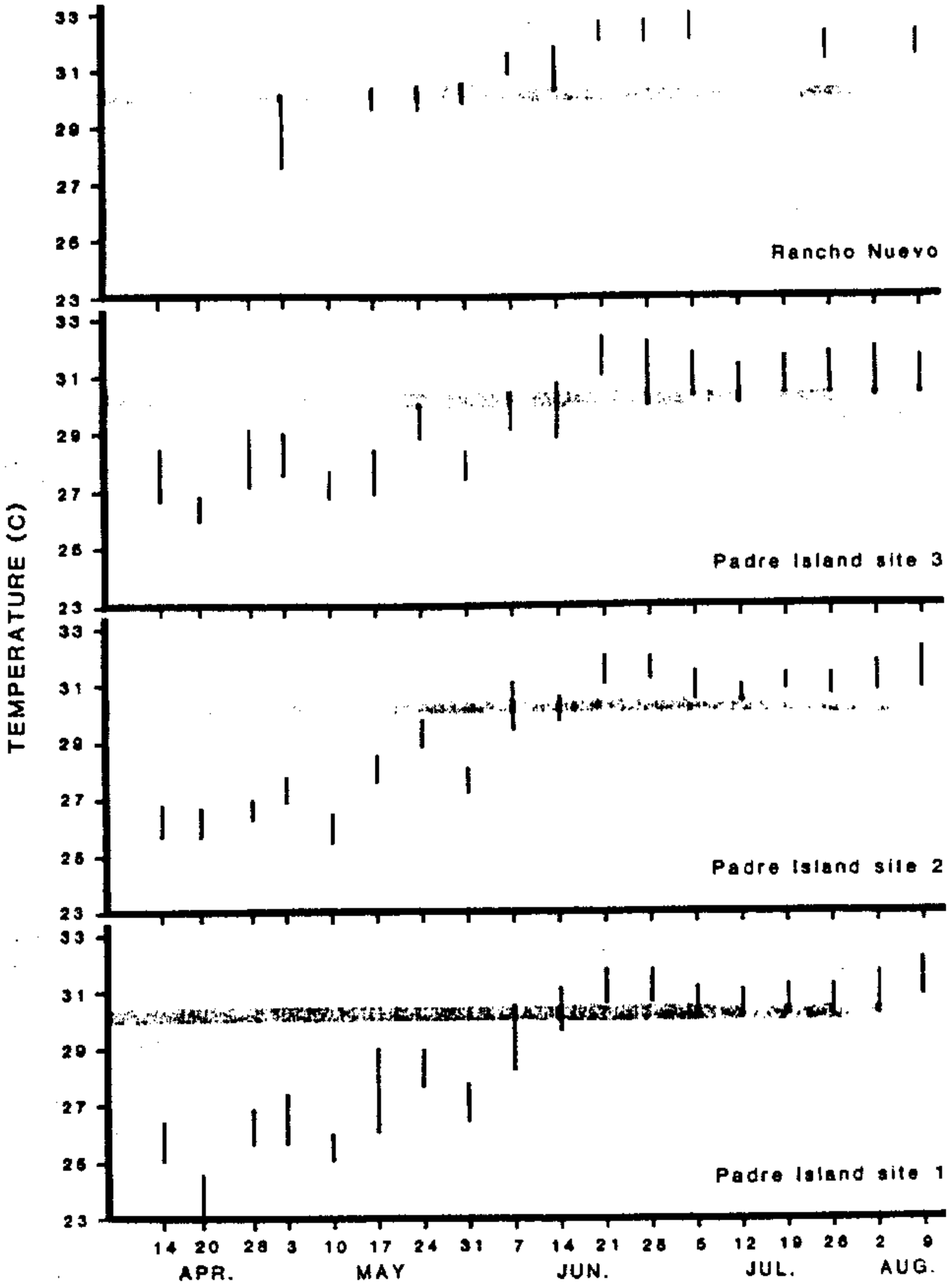

Figure 2. Range of mean 30 cm depth sand temperatures recorded from mid-beach to the middle upslope of the second foredune at three Padre Island National Seashore sites and one Rancho Nuevo site. Shaded area represents the estimated pivotal temperature and 95% confidence intervals.

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